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Real-time activity-dependent drug microinjection

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Real-time (RT) software technology has an enormous potential to precisely control the spatio-temporal aspects of a stimulus and to build activity-dependent stimulus-response loops to interact with neural systems and control them in a millisecond time scale. Establishing these loops can be an essential step towards understanding the dynamics of many neural processes and can bridge between traditionally disparate levels of analysis. RT software technology has been previously exploited to build dynamic clamp protocols in electrophysiological preparations [1]. The same principles used in the dynamic-clamp technology can be generalized to develop new techniques of activity-dependent stimulation with applications in a broad spectrum of research in nervous systems [2]. Here we show how RT software technology can also be used to build protocols of activity-dependent real-time drug microinjection to stimulate neural systems.

The heart central pattern generator (CPG) from the cardiac ganglion of the crab *Carcinus maenas* was subjected to microinjections of GABA. The microinjections were delivered with a Picospritzer III, and the duration of the injection and the stimulation precise instant were controlled. Simultaneously, the membrane potential of one neuron was measured and an activity-dependent stimuli protocol of GABA microinjection was implemented with RT software technology.

Figure 1 shows the effect of activity-dependent GABA microinjection stimuli evoked by the real-time detection of three action potentials in a CPG neuron from the cardiac ganglion of *Carcinus maenas*. The top panel shows the control activity (irregular bursts with six or more spikes). The middle panel shows the beginning of a RT stimulation protocol that consists of GABA injection when more than two spikes are detected (the vertical arrows indicate the instant in which the microinjection takes place). The bottom panel shows the activity a few seconds after the beginning of the protocol. This stimulation protocol was

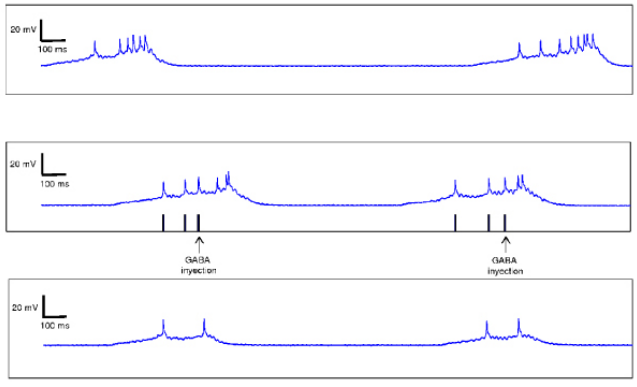

Figure 1

able to regularize the burst activity and maintain this activity with only two spikes in every burst without periodic injections of GABA.

We have illustrated a simple protocol of real-time event-driven drug microinjection to achieve a desired state in the spiking-bursting activity of CPG neurons. Real-time software technology allows to implement activity-dependent drug microinjection that can be applied to the study of many aspects of neuromodulation and neurotransmitter stimulation, and to achieve control of pathological states through temporal precise release of drugs.

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